Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	5354	cgmp or cyclic gmp	US-PGPUB; USPAT	ADJ	OFF	2005/02/06 11:37
L2	244	1 near5 (detect$ or indicat$)	US-PGPUB; USPAT	ADJ	OFF	2005/02/06 11:38
L3	5	2 same (fluorescen$ or fret)	US-PGPUB; USPAT	ADJ	OFF	2005/02/06 11:38
L4	17	1 near5 (fluorescen$ or fret)	US-PGPUB; USPAT	ADJ	OFF	2005/02/06 11:44
L5	224	1 same (fluorescen$ or fret)	US-PGPUB; USPAT	ADJ	OFF	2005/02/06 11:47
L6	12	2 and 5	US-PGPUB; USPAT	ADJ	OFF	2005/02/06 11:47

Priority to 7/4/00

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	5354	cgmp or cyclic gmp	US-PGPUB; USPAT	ADJ	OFF	2005/02/06 11:37
L2	244	1 near5 (detect$ or indicat$)	US-PGPUB; USPAT	ADJ	OFF	2005/02/06 11:38
L3	5	2 same (fluorescen$ or fret)	US-PGPUB; USPAT	ADJ	OFF	2005/02/06 11:38

PGPUB-DOCUMENT-NUMBER: 20040219523

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040219523 A1

TITLE: Nucleic acid sensor molecules and methods of using same

PUBLICATION-DATE: November 4, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Stanton, Martin	Stow	MA	US	
Epstein, David	Belmont	MA	US	
Hamaguchi, Nobuko	Framingham	MA	US	
Kurz, Markus	Newton	MA	US	
Keefe, Tony	Cambridge	MA	US	
Wilson, Charles	Concord	MA	US	
Grate, Dilara	Waltham	MA	US	
Marshall, Kristin A.	Arlington	MA	US	
McCauley, Thomas G.	Somerville	MA	US	
Kurz, Jeffrey C.	Somerville	MA	US	

APPL-NO: 10/ 215982

DATE FILED: August 9, 2002

RELATED-US-APPL-DATA:

child 10215982 A1 20020809

parent continuation-in-part-of 09952680 20010913 US ABANDONED

non-provisional-of-provisional 60311378 20010809 US

non-provisional-of-provisional 60313932 20010821 US

non-provisional-of-provisional 60338186 20011113 US

non-provisional-of-provisional 60349959 20020118 US

non-provisional-of-provisional 60364486 20020313 US

non-provisional-of-provisional 60367991 20020325 US

non-provisional-of-provisional 60369887 20020404 US

non-provisional-of-provisional 60376744 20020501 US

non-provisional-of-provisional 60385097 20020531 US

non-provisional-of-provisional 60232454 20000913 US

US-CL-CURRENT: 435/6, 536/23.1

ABSTRACT:

Methods for engineering a nucleic acid sensor molecule are provided.

Biosensors comprise a plurality of nucleic acid sensor molecules labeled with a first signaling moiety and a second signaling moiety. The nucleic acid sensor molecules recognizes target molecules which do not naturally bind to DNA. Binding of a target molecule to the sensor molecules triggers a change in the proximity of the signaling moieties which leads to a change in the optical properties of the nucleic acid sensor molecules on the biosensor. Reagents and systems for performing the method are also provided. The method is useful in diagnostic applications and drug optimization.

RELATED APPLICATIONS

[0001] This application claims priority to U.S. Ser. No. 60/311,378, filed Aug. 9, 2001; U.S. Ser. No. 60/313,932, filed Aug. 21, 2001; U.S. Ser. No. 60/338,186, filed Nov. 13, 2001; U.S. Ser. No. 60/349,959, filed Jan. 18, 2002; U.S. Ser. No. 60/364,486, filed Mar. 13, 2002; U.S. Ser. No. 60/367,991, filed Mar. 25, 2002; U.S. Ser. No. 60/369,887, filed April 4, 2002; U.S. Ser. No. 60/376,744, filed May 1, 2002; U.S. Ser. No. 60/385,097, filed May 31, 2002; U.S. Ser. No. 60/391,719, filed Jun. 26, 2002; and U.S. Ser. No. 09/952,680, filed Sep. 13, 2001, which claims priority to U.S. Ser. No. 60/232,454, filed Sep. 13, 2000. The contents of these applications are incorporated herein by reference in their entirety.

---------- KWIC --------

Brief Description of Drawings Paragraph - DRTX (71):

[0136] FIGS. 65A and 65B compare the observed pseudo-first order rate constants from solution- and solid-phase FRET sensor constructs. FIG. 65 C, D, and E shows experimental data and constructs for multiplexed detection using solution-phase cGMP and cAMP FRET.

Detail Description Paragraph - DETX (604):

[0730] This cGMP-dependent hammerhead NASM system was immobilized on streptavidin-impregnated membranes, and target-activated FRET activity observed. The generalization of this application of surface-immobilized NASM with FRET detection to a micro- or macro-arrayed format on an extended substrate such as glass or plastic is easily envisioned. Such a sensor array could be used to detect and quantify the presence of an arbitrary target molecule in a complex solution, e.g., crude cell extract or biological fluid, in real time. In addition, this general NASM strategy could be extended to accomplish multiplexed detection of multiple analytes in a sample simultaneously, by using NASMs labeled with fluorophores having different emission wavelengths. Experimental data for multiplexed detection using solution-phase cGMP and cAMP FRET sensors is shown in FIG. 66B. This NASM, and further extensions of it to include large numbers of unique analyte-sensors, could be used for high throughput screening (HTS) in drug discovery or clonal analysis. In all of these scenarios, optical detection of the FRET signals could be accomplished using a commercially available microarray imager or scanning fluorescence microscope.

US-PAT-NO: 6790629

DOCUMENT-IDENTIFIER: US 6790629 B2

TITLE: Nucleic acid sequences encoding capsaicin receptor and capsaicin receptor-related polypeptides and uses thereof

DATE-ISSUED: September 14, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Julius; David J.	San Francisco	CA	N/A	N/A
Caterina; Michael J.	Mill Valley	CA	N/A	N/A
Brake; Anthony J.	Berkeley	CA	N/A	N/A

APPL-NO: 09/ 978303

DATE FILED: October 15, 2001

PARENT-CASE:

CROSS-REFERENCES TO RELATED APPLICATIONS

This application is a continuation of U.S. patent application Ser. No. 09/235,451, filed Jan. 22, 1999, now U.S. Pat. No. 6,335,180, which is a continuation-in-part of: 1) U.S. Provisional patent application Ser. No. 60/072,151, filed Jan. 22, 1998; and 2) U.S. patent application Ser. No. 08/915,461, filed Aug. 20, 1997, now abandoned; and 3) PCT international application PCT/US98/17466, filed Aug. 20, 1998, which PCT International application was published in English on Jul. 29, 1999 as WO 9937675, each of which applications are incorporated herein by reference.

US-CL-CURRENT: 435/7.21, 435/7.1

ABSTRACT:

The present invention features vanilloid receptor polypeptides and vanilloid receptor-related polypeptides, specifically the capsaicin receptor subtypes VR1 and VR2 (VRRP-1), as well as the encoding polynucleotide sequences. In related aspects the invention features expression vectors and host cells comprising such polynucleotides. In other related aspects, the invention features transgenic animals having altered capsaicin receptor expression, due to, for example, the presence of an exogenous wild-type or modified capsaicin receptor-encoding polynucleotide sequence. The present invention also relates to antibodies that bind specifically to a capsaicin receptor polypeptide, and methods for producing these polypeptides. Further, the invention provides methods for using capsaicin receptor, including methods for screening candidate agents for activity as agonists or antagonists of capsaicin receptor activity, as well as assays to determine the amount of a capsaicin receptor-activating agent in a sample. In other related aspects, the invention provides methods for the use of the capsaicin receptor for the diagnosis and treatment of human disease and painful syndromes.

15 Claims, 28 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 12

---------- KWIC --------

Detailed Description Text - DETX (96):

Preferably, capsaicin receptor-binding compounds are screened for agonistic or antagonist action in a functional assay that monitors a biological activity associated with capsaicin receptor function such as effects upon intracellular levels of cations in a capsaicin receptor-expressing host cell (e g., calcium, magnesium, guanidinium, cobalt, potassium, cesium, sodium, and choline, preferably calcium), ligand-activated conductances, cell death (i.e., receptor-mediated cell death which can be monitored using, e.g., morphological assays, chemical assays, or immunological assays), depolarization of the capsaicin receptor-expressing cells (e.g., using fluorescent voltage-sensitive dyes), second messenger production (e.g., through detection of changes in cyclic GMP levels (see, e.g., Wood et al. 1989 J. Neurochem. 53:1203-1211), which can be detected by radioimmunoassay or ELISA), calcium-induced reporter gene expression (see, e.g., Ginty 1997 Neuron 18:183-186), or other readily assayable biological activity associated with capsaicin receptor activity or inhibition of capsaicin receptor activity. Preferably, the functional assay is based upon detection of a biological activity of capsaicin receptor that can be assayed using high-throughput screening of multiple samples simultaneously, e.g., a functional assay based upon detection of a change in fluorescence which in turn is associated with a change in capsaicin receptor activity. Such functional assays can be used to screen candidate agents for activity as either capsaicin receptor agonists or antagonists.

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	5354	cgmp or cyclic gmp	US-PGPUB; USPAT	ADJ	OFF	2005/02/06 11:37
L2	244	1 near5 (detect$ or indicat$)	US-PGPUB; USPAT	ADJ	OFF	2005/02/06 11:38
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L4	17	1 near5 (fluorescen$ or fret)	US-PGPUB; USPAT	ADJ	OFF	2005/02/06 11:44

PGPUB-DOCUMENT-NUMBER: 20040266783

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040266783 A1

TITLE: Assay for phosphodiesterase function

PUBLICATION-DATE: December 30, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Corbin, Jackie D.	Nashville	TN	US	
Francis, Sharron H.	Nashvillle	TN	US	

APPL-NO: 10/ 824771

DATE FILED: April 15, 2004

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60462899 20030415 US

US-CL-CURRENT: 514/252.16, 435/21

ABSTRACT:

The present invention provides filter-based assays for measuring binding of compounds to phosphodiesterases (PDEs). The assay permits stoichiometric binding of compounds to PDEs, thereby providing a highly sensitive measure of a PDE binding and inhibition.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (8):

[0009] Negative feedback regulation of cGMP would be enhanced if cGMP binding to the GAF domain also directly stimulates the catalytic domain. This was predicted earlier from the principle of reciprocity (Thomas et al., 1990a; Weber, 1975; Francis et al., 1990). Binding of 3-isobutyl-1-methylxanthine (IBMX) or a similar ligand to the catalytic domain has been shown to stimulate binding of cGMP to a GAF domain, but direct evidence that binding of cGMP to a GAF domain stimulates the catalytic domain has been elusive. This has been due in large part to difficulties in performing such studies with PDE5, in which both the catalytic domain and GAF domain possess high specificity for cGMP. Okada & Asakawa recently reported that cGMP stimulates PDE5 catalytic activity when measured using a fluorescent cGMP analog that is specific for the catalytic site of the enzyme (Okada & Asakawa, 2002). They suggested that this stimulation occurs through cGMP binding to the GAF domains.

PGPUB-DOCUMENT-NUMBER: 20040219523

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040219523 A1

TITLE: Nucleic acid sensor molecules and methods of using same

PUBLICATION-DATE: November 4, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Stanton, Martin	Stow	MA	US	
Epstein, David	Belmont	MA	US	
Hamaguchi, Nobuko	Framingham	MA	US	
Kurz, Markus	Newton	MA	US	
Keefe, Tony	Cambridge	MA	US	
Wilson, Charles	Concord	MA	US	
Grate, Dilara	Waltham	MA	US	
Marshall, Kristin A.	Arlington	MA	US	
McCauley, Thomas G.	Somerville	MA	US	
Kurz, Jeffrey C.	Somerville	MA	US	

APPL-NO: 10/ 215982

DATE FILED: August 9, 2002

RELATED-US-APPL-DATA:

child 10215982 A1 20020809

parent continuation-in-part-of 09952680 20010913 US ABANDONED

non-provisional-of-provisional 60311378 20010809 US

non-provisional-of-provisional 60313932 20010821 US

non-provisional-of-provisional 60338186 20011113 US

non-provisional-of-provisional 60349959 20020118 US

non-provisional-of-provisional 60364486 20020313 US

non-provisional-of-provisional 60367991 20020325 US

non-provisional-of-provisional 60369887 20020404 US

non-provisional-of-provisional 60376744 20020501 US

non-provisional-of-provisional 60385097 20020531 US

non-provisional-of-provisional 60232454 20000913 US

US-CL-CURRENT: 435/6, 536/23.1

ABSTRACT:

Methods for engineering a nucleic acid sensor molecule are provided.

Biosensors comprise a plurality of nucleic acid sensor molecules labeled with a first signaling moiety and a second signaling moiety. The nucleic acid sensor molecules recognizes target molecules which do not naturally bind to DNA. Binding of a target molecule to the sensor molecules triggers a change in the proximity of the signaling moieties which leads to a change in the optical properties of the nucleic acid sensor molecules on the biosensor. Reagents and systems for performing the method are also provided. The method is useful in diagnostic applications and drug optimization.

RELATED APPLICATIONS

[0001] This application claims priority to U.S. Ser. No. 60/311,378, filed Aug. 9, 2001; U.S. Ser. No. 60/313,932, filed Aug. 21, 2001; U.S. Ser. No. 60/338,186, filed Nov. 13, 2001; U.S. Ser. No. 60/349,959, filed Jan. 18, 2002; U.S. Ser. No. 60/364,486, filed Mar. 13, 2002; U.S. Ser. No. 60/367,991, filed Mar. 25, 2002; U.S. Ser. No. 60/369,887, filed April 4, 2002; U.S. Ser. No. 60/376,744, filed May 1, 2002; U.S. Ser. No. 60/385,097, filed May 31, 2002; U.S. Ser. No. 60/391,719, filed Jun. 26, 2002; and U.S. Ser. No. 09/952,680, filed Sep. 13, 2001, which claims priority to U.S. Ser. No. 60/232,454, filed Sep. 13, 2000. The contents of these applications are incorporated herein by reference in their entirety.

---------- KWIC ---------

Brief Description of Drawings Paragraph - DRTX (71):

[0136] FIGS. 65A and 65B compare the observed pseudo-first order rate constants from solution- and solid-phase FRET sensor constructs. FIG. 65 C, D, and E shows experimental data and constructs for multiplexed detection using solution-phase cGMP and cAMP FRET.

Detail Description Paragraph - DETX (391):

[0536] The nucleotide sequences of a cAMP-dependent PDE nucleic acid sensor molecule and cGMP-dependent nucleic acid sensor molecule are presented in Table 10. Allosteric domains are 15 shown in bold font and the cleavage site nucleotide is underlined. CGMP modulated NASMs that are configured for homogeneous, solution based fluorescence assays (FRET) are shown the FIG. 62. Multiplexed camp and cGMP-modulated FRET-sensor NASM-based assays are shown in FIG. 65B. The optical NASMs modulated by cAMP and cGMP are used in PDE assays as described in detail below.

Detail Description Paragraph - DETX (602):

[0728] This target-activated nucleic acid sensor molecule system constitutes a highly sensitive real-time sensor for detecting and quantitating the concentration of the target molecule present in an unknown sample solution. The ultimate limit of detection (LOD) for this system is determined by the switch factor, defined as the ratio of the catalytic rate (in this example, the rate of cleavage) of the ribozyme sensor in the presence of its target to that of the ribozyme in the absence of its target. The dynamic range of the ribozyme sensor will be determined by the dissociation constant, K.sub.d, for the interaction of the ribozyme binding domain with the target molecule. In theory, the effective dynamic range over which the rate-response of the NASM is linear in the target concentration has K.sub.d as an upper bound. The lower panel of FIG. 63 shows experimental data from the surface-immobilized cGMP-activated NASM. The data shown in the figure represents the FRET signal from the donor fluorophore with the sensor exposed to concentrations of 0, 100 uM, and 600 uM cGMP. The upper panel of FIG. 64 shows both donor and acceptor fluorescence signals for the FRET system in the presence of 200 uM cGMP. Note

that the experimental data exhibits the behavior expected, as shown in the lower panel of FIG. 63. The lower panel of FIG. 64 shows the donor signal form the plot in the upper panel fitted to a pseudo-first order rate equation. As shown by the closeness of the data fit, the kinetic response of this sensor system closely approximates a pseudo-first order reaction.

Detail Description Paragraph - DETX (604):

[0730] This cGMP-dependent hammerhead NASM system was immobilized on streptavidin-impregnated membranes, and target-activated FRET activity observed. The generalization of this application of surface-immobilized NASM with FRET detection to a micro- or macro-arrayed format on an extended substrate such as glass or plastic is easily envisioned. Such a sensor array could be used to detect and quantify the presence of an arbitrary target molecule in a complex solution, e.g., crude cell extract or biological fluid, in real time. In addition, this general NASM strategy could be extended to accomplish multiplexed detection of multiple analytes in a sample simultaneously, by using NASMs labeled with fluorophores having different emission wavelengths. Experimental data for multiplexed detection using solution-phase cGMP and cAMP FRET sensors is shown in FIG. 66B. This NASM, and further extensions of it to include large numbers of unique analyte-sensors, could be used for high throughput screening (HTS) in drug discovery or clonal analysis. In all of these scenarios, optical detection of the FRET signals could be accomplished using a commercially available microarray imager or scanning fluorescence microscope.

PGPUB-DOCUMENT-NUMBER: 20040203081

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040203081 A1

TITLE: Natriuretic compounds, conjugates, and uses thereof

PUBLICATION-DATE: October 14, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
James, Kenneth D.	Mebane	NC	US	
Radhakrishnan, Balasingham	Chapel Hill	NC	US	
Malkar, Navdeep B.	Cary	NC	US	
Miller, Mark A.	Raleigh	NC	US	
Ekwuribe, Nnochiri N.	Cary	NC	US	

APPL-NO: 10/ 723933

DATE FILED: November 26, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60429151 20021126 US

US-CL-CURRENT: 435/7.23, 424/155.1 , 530/388.8

ABSTRACT:

Modified n atriuretic compounds and conjugates thereof are disclosed in the present invention. In particular, conjugated forms of hBNP are provided that include at least one modifying moiety attached thereto. The modified natriuretic compound conjugates retain activity for stimulating cGMP production, binding to NPR-A receptor, and in some embodiments an improved half-life in circulation as compared to unmodified counterpart natriuretic compounds. Oral, parenteral, subcutaneous, and intravenous forms of the compounds and conjugates may be prepared as treatments and/or therapies for heart conditions particularly congestive heart failure. Modifying moieties comprising oligomeric structures having a variety of lengths and configurations are also disclosed. Analogs of the n atriuretic compound are also disclosed, having an amino acid sequence that is other than the native sequence.

1. CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is based on and claims priority to U.S. Provisional Application Serial No. 60/429,151, filed Nov. 26, 2002, the entire contents of which is herein incorporated by reference.

---------- KWIC ---------

Detail Description Paragraph - DETX (325):
[0356] Primary HAEC were purchased from Clonetics for cGMP screening. Cells were plated into 12 well plates the day before the experiment. On the day of the experiment, cells were pre-incubated for 10 min at 37.degree. C. with 0.5 mM IFBMX to inhibit phosphodiesterases. Test compounds were added to the cells for an additional 60 min at 37.degree. C. and the incubation was stopped by

lysing cells to measure cGMP. An ELISA-based cGMP kit was used to measure cGMP production (CatchPoint-cyclic GMP Fluorescent Assay Kit, catalog #R8074, Molecular Devices Corp, Sunnyvale, Calif.). This kit measures cGMP via a competitive immunoassay in 96-well format. Cell lysates were added to the coated microplate followed by the addition of an anti-cGMP antibody and a horseradish peroxidase (HRP)-cGMP conjugate. Plates were incubated for two hours at room temperature, followed by four washes. A substrate solution was added and the fluorescent intensity of each well was quantitated. The fluorescent signal intensity decreased with increasing levels of cGMP. Native hBNP was be tested in each experiment as a positive control.

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	5354	cgmp or cyclic gmp	US-PGPUB; USPAT	ADJ	OFF	2005/02/06 11:37
L2	244	1 near5 (detect$ or indicat$)	US-PGPUB; USPAT	ADJ	OFF	2005/02/06 11:38
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L5	224	1 same (fluorescen$ or fret)	US-PGPUB; USPAT	ADJ	OFF	2005/02/06 11:47
L6	12	2 and 5	US-PGPUB; USPAT	ADJ	OFF	2005/02/06 11:47

PGPUB-DOCUMENT-NUMBER: 20040229251

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040229251 A1

TITLE: Detection of guanylyl and adenylyl cyclase activity

PUBLICATION-DATE: November 18, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Sunahara, Roger K.	Ann Arbor	MI	US	
Neubig, Richard R.	Ann Arbor	MI	US	

APPL-NO: 10/ 779404

DATE FILED: February 13, 2004

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60447074 20030213 US

US-CL-CURRENT: 435/6, 435/199

ABSTRACT:

The present invention relates to methods of assaying nucleotide cyclase activity. In particular, the present invention relates to fluorescence-based methods of assaying guanylyl and adenylyl cyclase activity.

[0001] This application claims priority to provisional patent application serial No. 60/447,074, filed Feb. 13, 2003, which is herein incorporated by reference in its entirety.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (6):

[0006] Currently two methods are commonly used to measure guanylyl and adenylyl cyclase activity. One method utilizes radioactive GTP (or ATP) and measures the accumulation of radioactive cGMP (or cAMP), while the other method uses a radioimmunoassay procedure (RIA) to detect cGMP (or cAMP). For example, one assay measures the conversion of radioactive GTP (or ATP) to radioactive cGMP (or cAMP). For intact cell studies the conversion of metabolically-labeled [.sup.3H]GTP or [.sup.3H]ATP, derived from whole cells assays incubated with [.sup.3H]guanine, or [3H]adenine) to [.sup.3H]cGMP (or [.sup.3H]cAMP) is measured. For broken cell assays or assays with purified GC protein, the conversion of [.sup.32P].alpha.GTP to [.sup.32P]cGMP, or [.sup.32P].alpha.ATP to [.sup.32P]cAMP, is measured. Following activation of the enzyme, the reaction is terminated by acid treatment, SDS solublization or by heat inactivation. In either case radioactive cGMP is separated from other radioactive species, a lengthy process that involves multiple chromatography steps. Another method measures non-radioactive cGMP levels using antibodies against cGMP in a radioimmunoassay (RIA). This process requires lengthy incubations and still requires the use of radioactivity, usually

[.sup.125I]cGMP, as a tracer.

Summary of Invention Paragraph - BSTX (7):
[0007] These methods utilize lengthy protocols all requiring the use of radioactive compounds. GC assays using either protocol require several hours to obtain results and data cannot be attained in a real-time format. Furthermore, multiple transfers during the isolation of cGMP and scintillation counting, Cerenkov detection, or gamma radiation detection are required. All of these processes result in significant accumulation of radioactive wastes. In order to screen potential AC and GC ligands and modulators, additional screening methods are needed. Preferred assays are those suitable for high throughput screening.

Detail Description Paragraph - DETX (38):
[0054] In some embodiments, the present invention provides a cyclase activity assay that utilizes a fluorescently labeled nucleotide triphosphate (e.g., BODIPY FL-GTP.gamma.S). The intrinsic fluorescence of BODIPY FL-GTP.gamma.S has been previously been shown to increase stiochiometrically with binding to guanine-nucleotide binding proteins (G proteins) (McEwen et al., Methods in Enzymology 344:403-20 [2002]; McEwen et al., Analytical Biochemistry 291:109-17 [2001]). The present invention is not limited to a particular mechanism of action. Indeed, an understanding of the mechanism is not necessary to practice the present invention. Nonetheless, it is contemplated that fluorescence increase occurs as a result of removal of the quenching effect of the purine ring of GTP on the BODIPY moiety. Binding to G proteins relieves the ring-stacking effect and unquenches the fluorophore. The formation of cGMP involves ligation of the 3'OH of the ribose ring of GTP to the alpha phosphate with PPi, the beta- and gamma-phosphates, serving as the leaving group. The reaction is not dramatically affected by subtle substitutions on the gamma phosphate, such as a thio moiety.

Detail Description Paragraph - DETX (39):
[0055] The gamma-thio forms of GTP (e.g., GTP.gamma.S) are not natural substrates for G proteins. GTP.gamma.S is however a substrate of GCs though cleavage of the ester bond between the alpha and beta phosphates. Experiments conducted during the course of development of the present invention (Example 1) demonstrated that BODIPY FL-GTP.gamma.S is also a substrate for GC and displays increasing fluorescence corresponding to increasing GC activity. BODIPY-GTP.gamma.S hydrolyzes to cGMP and the unquenched BODIPY-conjugated thio-pyrophosphate.

PGPUB-DOCUMENT-NUMBER: 20040219523

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040219523 A1

TITLE: Nucleic acid sensor molecules and methods of using same

PUBLICATION-DATE: November 4, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Stanton, Martin	Stow	MA	US	
Epstein, David	Belmont	MA	US	
Hamaguchi, Nobuko	Framingham	MA	US	
Kurz, Markus	Newton	MA	US	
Keefe, Tony	Cambridge	MA	US	
Wilson, Charles	Concord	MA	US	
Grate, Dilara	Waltham	MA	US	
Marshall, Kristin A.	Arlington	MA	US	
McCauley, Thomas G.	Somerville	MA	US	
Kurz, Jeffrey C.	Somerville	MA	US	

APPL-NO: 10/ 215982

DATE FILED: August 9, 2002

RELATED-US-APPL-DATA:

child 10215982 A1 20020809

parent continuation-in-part-of 09952680 20010913 US ABANDONED

non-provisional-of-provisional 60311378 20010809 US

non-provisional-of-provisional 60313932 20010821 US

non-provisional-of-provisional 60338186 20011113 US

non-provisional-of-provisional 60349959 20020118 US

non-provisional-of-provisional 60364486 20020313 US

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non-provisional-of-provisional 60369887 20020404 US

non-provisional-of-provisional 60376744 20020501 US

non-provisional-of-provisional 60385097 20020531 US

non-provisional-of-provisional 60232454 20000913 US

US-CL-CURRENT: 435/6, 536/23.1

ABSTRACT:

Methods for engineering a nucleic acid sensor molecule are provided.

Biosensors comprise a plurality of nucleic acid sensor molecules labeled with a first signaling moiety and a second signaling moiety. The nucleic acid sensor molecules recognizes target molecules which do not naturally bind to DNA. Binding of a target molecule to the sensor molecules triggers a change in the proximity of the signaling moieties which leads to a change in the optical properties of the nucleic acid sensor molecules on the biosensor. Reagents and systems for performing the method are also provided. The method is useful in diagnostic applications and drug optimization.

RELATED APPLICATIONS

[0001] This application claims priority to U.S. Ser. No. 60/311,378, filed Aug. 9, 2001; U.S. Ser. No. 60/313,932, filed Aug. 21, 2001; U.S. Ser. No. 60/338,186, filed Nov. 13, 2001; U.S. Ser. No. 60/349,959, filed Jan. 18, 2002; U.S. Ser. No. 60/364,486, filed Mar. 13, 2002; U.S. Ser. No. 60/367,991, filed Mar. 25, 2002; U.S. Ser. No. 60/369,887, filed April 4, 2002; U.S. Ser. No. 60/376,744, filed May 1, 2002; U.S. Ser. No. 60/385,097, filed May 31, 2002; U.S. Ser. No. 60/391,719, filed Jun. 26, 2002; and U.S. Ser. No. 09/952,680, filed Sep. 13, 2001, which claims priority to U.S. Ser. No. 60/232,454, filed Sep. 13, 2000. The contents of these applications are incorporated herein by reference in their entirety.

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Summary of Invention Paragraph - BSTX (54):
[0052] In still another aspect, the invention provides a nucleic acid sensor molecule which includes a target modulation domain that recognizes any one of cCMP, cAMP, or cGMP, a catalytic domain, and a linker domain that links the target modulation domain and the catalytic domain, wherein the nucleic acid sensor molecule includes an optical signal generating unit or a non-radioactive detectable label. In some embodiments, the nucleic acid sensor molecule includes an optical signal generating unit. In other embodiments, the nucleic acid sensor molecule includes a detectable label. In a particular embodiment, the label is a radioactive label, such as, for example, .sup.32P, .sup.33P, .sup.14C, .sup.35S, .sup.3H, or .sup.125I. In other embodiments, the nucleic acid sensor molecule comprises a fluorescent label, such as, for example, fluorescein, DABCYL, or a green fluorescent protein (GFP) moiety. In some embodiments, the optical signal generating unit includes a fluorescent moiety and a quenching moiety, wherein recognition by the target modulation domain causes causes a change in detectable fluorescence by the optical signal generating unit. In some embodiments, the nucleic acid sensor molecule includes an enzymatic label, or an affinity capture tag label.

Brief Description of Drawings Paragraph - DRTX (68):
[0133] FIG. 62 shows the original solution-phase cGMP-dependent hammerhead nucleic acid sensor molecule FRET construct (SEQ ID NO:101) and its effector/capture oligo (SEQ ID NO:102) from which the solid-phase FRET sensor was derived. In the solution-phase construct shown in FIG. 62A, the fluorophore (F) and quencher (Q) are FAM and DABCYL, respectively. In the solid-phase construct shown in FIG. 62B, the donor fluorophore (D) and acceptor fluorophore (A) are FAM and AlexaFluor 568, respectively.

Brief Description of Drawings Paragraph - DRTX (69):
[0134] FIG. 63A shows the surface-immobilized FRET sensor before, and FIG. 63B shows after, exposure to the activating target molecule (cGMP), followed by subsequent cleavage and dissociation of the sequence fragment containing the

acceptor fluorophore (A). FIG. 63C shows the expected kinetic time course signals and FIG. 63D shows the actual kinetic time course signals observed from these sensors in the presence of various concentrations of target.

Brief Description of Drawings Paragraph - DRTX (70):

[0135] FIG. 64 shows fitted kinetic time course signals observed from the solid-phase FRET sensor constructs in a solution-phase assay. FIG. 64A shows a graph that plots the signal observed from the donor fluorophore only in the presence of 200 uM cGMP. FIG. 64B shows a graph of the parametric fit to the experimental data shown in FIG. 64A, verifying that the rate constant for the solid-phase construct is in fact similar to that for the solution-phase construct under similar conditions.

Brief Description of Drawings Paragraph - DRTX (71):

[0136] FIGS. 65A and 65B compare the observed pseudo-first order rate constants from solution- and solid-phase FRET sensor constructs. FIG. 65 C, D, and E shows experimental data and constructs for multiplexed detection using solution-phase cGMP and cAMP FRET.

Detail Description Paragraph - DETX (391):

[0536] The nucleotide sequences of a cAMP-dependent PDE nucleic acid sensor molecule and cGMP-dependent nucleic acid sensor molecule are presented in Table 10. Allosteric domains are 15 shown in bold font and the cleavage site nucleotide is underlined. CGMP modulated NASMs that are configured for homogeneous, solution based fluorescence assays (FRET) are shown the FIG. 62. Multiplexed camp and cGMP-modulated FRET-sensor NASM-based assays are shown in FIG. 65B. The optical NASMs modulated by cAMP and cGMP are used in PDE assays as described in detail below.

Detail Description Paragraph - DETX (597):

[0723] This example describes a general method for implementing a FRET-based (fluorescence resonance energy transfer) assay utilizing nucleic acid sensor molecules. (in this case, cGMP-dependent hammerhead nucleic acid sensor molecule) wherein the nucleic acid sensor molecule is immobilized on a solid substrate, e.g., within a microtiter plate well, on a membrane, on a glass or plastic microscope slide, etc.

Detail Description Paragraph - DETX (600):

[0726] As shown in FIG. 62 (lower panel), the donor and acceptor fluorophores form an efficient FRET-pair; that is, upon excitation of the donor fluorophore near its spectral absorption maxima, the incident electromagnetic energy is efficiently transferred (nonradiatively) via resonant electric dipole coupling from the donor fluorophore to the acceptor fluorophore. The efficiency of this resonant energy transfer is strongly dependent on the separation between the donor and acceptor fluorophores, the transfer rate being proportional to 1/R.sup.6, where R is the intermolecular separation. Therefore, when the donor and acceptor are in close proximity, i.e., a few bond-lengths or roughly 10-50 Angstroms, the fluorescent emission from donor species will be reduced relative to its output in an isolated configuration, while the emission from the acceptor species, through indirect excitation by the donor, will be detectable. Upon separation of the donor and acceptor, the donor fluorescence emission signal will increase strongly, while the acceptor emission signal will show a commensurate decrease in intensity. These effects are shown in FIG. 63 (upper panel) for the cGMP-dependent NASM system (SEQ ID: NO. 102).

Detail Description Paragraph - DETX (602):

[0728] This target-activated nucleic acid sensor molecule system constitutes a highly sensitive real-time sensor for detecting and quantitating the concentration of the target molecule present in an unknown sample solution. The ultimate limit of detection (LOD) for this system is determined by the switch factor, defined as the ratio of the catalytic rate (in this example, the rate of cleavage) of the ribozyme sensor in the presence of its target to that of the ribozyme in the absence of its target. The dynamic range of the ribozyme sensor will be determined by the dissociation constant, K.sub.d, for the interaction of the ribozyme binding domain with the target molecule. In theory, the effective dynamic range over which the rate-response of the NASM is linear in the target concentration has K.sub.d as an upper bound. The lower panel of FIG. 63 shows experimental data from the surface-immobilized cGMP-activated NASM. The data shown in the figure represents the FRET signal from the donor fluorophore with the sensor exposed to concentrations of 0, 100 uM, and 600 uM cGMP. The upper panel of FIG. 64 shows both donor and acceptor fluorescence signals for the FRET system in the presence of 200 uM cGMP. Note that the experimental data exhibits the behavior expected, as shown in the lower panel of FIG. 63. The lower panel of FIG. 64 shows the donor signal form the plot in the upper panel fitted to a pseudo-first order rate equation. As shown by the closeness of the data fit, the kinetic response of this sensor system closely approximates a pseudo-first order reaction.

Detail Description Paragraph - DETX (603):

[0729] The measured dissociation constant for this cGMP-activated NASM with cGMP is approximately 200-500 uM. In practice, concentration measurements up to 1 mM are possible with this sensor in solution-phase measurements. The absolute precision of measurements made with this NASM will depend on the amount of background catalytic activity (i.e., in the absence of target) and baseline drift of the fluorescence signals from both sample and controls due to physical factors, such as liquid handling errors, reagent adhesion, evaporation, or mixing. After some optimization, run-to-run CVs of a few percent are possible with similar FRET-based NASMs measured in solution. Immobilization of the NASM does not degrade its catalytic activity, although it may limit the effective availability of the target-binding domain for interaction with target molecules. The locally high concentration of surface-immobilized NASM will tend to offset this effect by driving the equilibrium for the association (and subsequent catalytic) reactions toward formation of ribozyme-target complex. The net result is a reduction in the observed catalytic rate of approximately 4-fold at K.sub.d (200-500 uM) for the surface-immobilized cGMP-dependent hammerhead NASM, relative to the observed rate for the same sensor system in solution. This effect is shown in the experimental data presented in FIG. 65A, which shows linear (Panel A) and logarithmic (Panel B) plots of the observed catalytic rate constant versus cGMP concentration for the cGMP-dependent hammerhead NASM in both solution and surface-immobilized ('solid') configurations. The upper panel plots the observed rate as a function of target (cGMP) concentration on linear axes, while the lower plot plots the same rate data on log-log axes. The observed rate constant (at a given concentration) for the immobilized solid-phase sensor is roughly 10-fold lower than that for the solution-phase sensor. The practical effect of this is that, while the solution-phase sensor's linear dynamic range is limited to about 0-600 uM, the solid-phase sensor's linear dynamic range is at least 2 mM. FIGS. 65C, 65D, and 65E shows how this general NASM strategy could be extended to accomplish multiplexed detection of multiple analytes in a sample simultaneously, by using NASMs labeled with fluorophores having different emission wavelengths. Squares are NASMs immobilized on a solid. Circles are NASMs in solution. In this case, the sensors were immobilized in neutravidin-coated microtiter plate wells (96 well plate, volume of 50 uL). Detection of the fluorescent signals was accomplished in this case by a microplate fluorescence reader equipped with the appropriate lamps,

optics, filters, and optical detectors (PMT) manufactured by Packard Instrument Co.

Detail Description Paragraph - DETX (604):

[0730] This cGMP-dependent hammerhead NASM system was immobilized on streptavidin-impregnated membranes, and target-activated FRET activity observed. The generalization of this application of surface-immobilized NASM with FRET detection to a micro- or macro-arrayed format on an extended substrate such as glass or plastic is easily envisioned. Such a sensor array could be used to detect and quantify the presence of an arbitrary target molecule in a complex solution, e.g., crude cell extract or biological fluid, in real time. In addition, this general NASM strategy could be extended to accomplish multiplexed detection of multiple analytes in a sample simultaneously, by using NASMs labeled with fluorophores having different emission wavelengths. Experimental data for multiplexed detection using solution-phase cGMP and cAMP FRET sensors is shown in FIG. 66B. This NASM, and further extensions of it to include large numbers of unique analyte-sensors, could be used for high throughput screening (HTS) in drug discovery or clonal analysis. In all of these scenarios, optical detection of the FRET signals could be accomplished using a commercially available microarray imager or scanning fluorescence microscope.

US-PAT-NO: 6790629

DOCUMENT-IDENTIFIER: US 6790629 B2

TITLE: Nucleic acid sequences encoding capsaicin receptor and capsaicin receptor-related polypeptides and uses thereof

DATE-ISSUED: September 14, 2004

INVENTOR-INFORMATION:

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APPL-NO: 09/ 978303

DATE FILED: October 15, 2001

PARENT-CASE:

CROSS-REFERENCES TO RELATED APPLICATIONS

This application is a continuation of U.S. patent application Ser. No. 09/235,451, filed Jan. 22, 1999, now U.S. Pat. No. 6,335,180, which is a continuation-in-part of: 1) U.S. Provisional patent application Ser. No. 60/072,151, filed Jan. 22, 1998; and 2) U.S. patent application Ser. No. 08/915,461, filed Aug. 20, 1997, now abandoned; and 3) PCT international application PCT/US98/17466, filed Aug. 20, 1998, which PCT International application was published in English on Jul. 29, 1999 as WO 9937675, each of which applications are incorporated herein by reference.

US-CL-CURRENT: 435/7.21, 435/7.1

ABSTRACT:

The present invention features vanilloid receptor polypeptides and vanilloid receptor-related polypeptides, specifically the capsaicin receptor subtypes VR1 and VR2 (VRRP-1), as well as the encoding polynucleotide sequences. In related aspects the invention features expression vectors and host cells comprising such polynucleotides. In other related aspects, the invention features transgenic animals having altered capsaicin receptor expression, due to, for example, the presence of an exogenous wild-type or modified capsaicin receptor-encoding polynucleotide sequence. The present invention also relates to antibodies that bind specifically to a capsaicin receptor polypeptide, and methods for producing these polypeptides. Further, the invention provides methods for using capsaicin receptor, including methods for screening candidate agents for activity as agonists or antagonists of capsaicin receptor activity, as well as assays to determine the amount of a capsaicin receptor-activating agent in a sample. In other related aspects, the invention provides methods for the use of the capsaicin receptor for the diagnosis and treatment of human disease and painful syndromes.

15 Claims, 28 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 12

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Detailed Description Text - DETX (96):

Preferably, capsaicin receptor-binding compounds are screened for agonistic or antagonist action in a functional assay that monitors a biological activity associated with capsaicin receptor function such as effects upon intracellular levels of cations in a capsaicin receptor-expressing host cell (e g., calcium, magnesium, guanidinium, cobalt, potassium, cesium, sodium, and choline, preferably calcium), ligand-activated conductances, cell death (i.e., receptor-mediated cell death which can be monitored using, e.g., morphological assays, chemical assays, or immunological assays), depolarization of the capsaicin receptor-expressing cells (e.g., using fluorescent voltage-sensitive dyes), second messenger production (e.g., through detection of changes in cyclic GMP levels (see, e.g., Wood et al. 1989 J. Neurochem. 53:1203-1211), which can be detected by radioimmunoassay or ELISA), calcium-induced reporter gene expression (see, e.g., Ginty 1997 Neuron 18:183-186), or other readily assayable biological activity associated with capsaicin receptor activity or inhibition of capsaicin receptor activity. Preferably, the functional assay is based upon detection of a biological activity of capsaicin receptor that can be assayed using high-throughput screening of multiple samples simultaneously, e.g., a functional assay based upon detection of a change in fluorescence which in turn is associated with a change in capsaicin receptor activity. Such functional assays can be used to screen candidate agents for activity as either capsaicin receptor agonists or antagonists.